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## SUMMARY OF TWO YEARS' STUDY OF INSECTS IN RELATION TO PELLAGRA

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With the growing interest in pellagra, following the authoritative recognition of its presence in the United States in 1907, the study of its etiology was taken up by various investigators and the several theories of causation were subjected to close scrutiny.

Prominent among these theories was that of insect transmission, first advanced by Sambon, who limited this function to the species of blood-sucking gnats comprising the genus *Simulium*.

The importance of the disease and the possibility of such a factor in its causation, led the Bureau of Entomology, late in 1911, to undertake an investigation of the subject in South Carolina, to which locality attention had been directed by the state authorities. The writer and W. V. King were, early in 1912, assigned by Dr. L. O. Howard, Chief of the Bureau of Entomology, United States Department of Agriculture, under the direction of Mr. W. D. Hunter of the Bureau, to investigate the possible relation of insects to pellagra and to gather such data as might serve to indicate whether there was ground for the assumption that blood-sucking or other arthropods were involved in the transmission of the disease in that region.

In June, 1912, the Thompson-McFadden Pellagra Commission of the Department of Tropical Medicine, New York Post-Graduate Medical School, established its laboratory and began its field work at Spartanburg, in Spartanburg County, S. C.

Through the courtesy of Capt. J. F. Siler, Medical Corps, United States Army, and with the approval of Dr. L. O. Howard, Chief of the Bureau of Entomology, the representatives of the Bureau were enabled to cooperate with the commission and to study the possible relation of insects to the causation of pellagra.

The work undertaken under these auspices consisted of a general study of such insects as appeared after a careful review of the situation to present possibilities in this connection. The species which seemed worthy of consideration were studied as to biology and habits, with special reference to the epidemiology of the disease and to the habits of those classes of the population in which appear the great mass of the cases of pellagra.

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The homes of pellagrins were studied, together with the sanitary condition of their surroundings, and especially careful attention was given to the mill villages in which occur many cases of the disease.

Collections of insects were made and no effort was spared to come to an unbiased conclusion in the case of each species studied. Careful elimination of such forms as failed to meet our conception of the characteristics required was effected on the grounds set forth below.

Field work was continued until October 15, and the details of the work was published as a progress report. In April, 1913, the work was again taken up. In June, a hospital for pellagrins having been established in Spartanburg, we were afforded facilities for more elaborate laboratory studies, including the attempted transmission of pellagra to monkeys by the bites of blood-sucking insects. The details of the latter work were carried on by Mr. King and will form the basis for a later report.

As a basis for our work it was necessary to assume that pellagra is an infectious disease and that it is capable of transmission by blood-sucking insects, but it must be well understood that no positive convictions were entertained and the possibility only of such conditions is implied. In weighing the evidence as to the involvement of each insect we were impelled to apply Knab's postulate in this connection, and I shall quote its substance:

In order to be a potential transmitter of human blood-parasites, an insect must be closely associated with man and normally have opportunity to suck his blood repeatedly. It is not sufficient that occasional specimens bite man as, for example, is the case with forest mosquitoes. Although a person may be bitten by a large number of such mosquitoes, the chance that any of these mosquitoes survive to develop the parasites in question (assuming such development to be possible), and then find opportunity to bite and infect another person, are altogether too remote.

The results of the work of 1913 were, in the main, corroborative of those of 1912, but further studies by the commission have broadened the view and introduced some new elements.

Two mill villages in counties adjacent to that of Spartanburg are equipped with an effective water-carriage system of sewage disposal. It was found that in these villages, pellagra has failed to obtain a foothold, although introduced a number of times. In the town of Spartanburg, also, there appears to be a correlation between the absence of sewers and the prevalence of pellagra.

The studies have also brought out the fact that a close domiciliary connection appears to exist between cases originating in 1912 and 1913, and older or antecedent cases; that is, the cases developing in these years, in the mill villages under consideration, which show a close household association with antecedent cases, form a large percentage of

the cases for three years. Cases which have been in less close association with older cases, such as neighbors living next door or across the street from them, form a much smaller percentage of the total. Those living at greater distances are, in some villages, wanting, the total number of such cases being very small.

That this phenomenon indicates infectiousness is so clear as to amount almost to a demonstration.

The prevalence of *Pediculus capitis* seems to be somewhat greater than we had supposed and we are led to believe that our previous views did not reflect the actual condition. This was due largely to the improved facilities for observation at our command in 1913 and partly also to the overcoming of reticence on the part of pellagra patients and their families.

The evidence gathered does not materially alter our views regarding the agency of these insects, but the fact that we had underestimated their abundance suggests the desirability of further work along this line in 1914.

The insects on which our studies were especially concentrated were the ticks, lice, bedbugs, roaches, horseflies (Tabanidae), fleas, mosquitoes, buffalo gnats (*Simulium*), houseflies and the stable fly (*Stomoxys calcitrans*).

The ticks (family Ixodidae, the only family of the group represented in South Carolina) can be safely excluded by reason of their biting habits and life history. The fact that most ticks of this family require three hosts during the life cycle, remain attached during each stage, drop to the ground at its completion and re-attach to another after molting, precludes their incrimination. So rarely could a tick remain attached to a human host a time sufficient for its engorgement and the completion of the current stage of its development, that its chances for becoming infective and living to transmit its infection would be practically *nil*. It must be borne in mind that we are dealing with a disease which shows no evidence of the existence of reservoirs of its virus among the lower animals. Ticks are not a serious pest in Spartanburg County, and those suffering most from pellagra, the home-keeping adult females and young children, are those least exposed to the bites of ticks.

The head louse was excluded by us in 1912 because its prevalence seemed inadequate to the dissemination of a disease with the epidemiologic characteristics of pellagra. The occurrence of a considerable number of cases among persons whose circumstances and habits should safeguard them from attack by *Pediculus* is opposed to the agency of the insects.

The relative incidence in males and females is not satisfactorily explained by their incrimination and the distribution of cases in the individual foci of the disease does not appear, in our opinion, strongly to indicate a flightless carrier with a human vehicle.

Notwithstanding our misconception as to the prevalence of the species, additional facts will be necessary to place it among the probable transmitters of pellagra.

In large cities, with congested populations, unhygienic surroundings and abundance of *Pediculus*, pellagra does not obtain a foothold in spite of the introduction of cases of the disease.

The bedbug, *Cimex lectularius*, although very abundant and universally distributed, when considered as a possible carrier of pellagra, does not account for certain marked characteristics of the disease. Its association with man is of the closest nature and the conditions under which a large class of pellagra suffers live, favor in high degree its indiscriminate attack on all members of the household generally. But the indiscriminate character of its attentions is the strongest argument against its incrimination.

Although the approximate ratio of infection of females to males, as a whole, in the United States is as 3 to 1, we find that among adults, nine women are victims of pellagra to every man affected. The ratio is much too high to be accounted for by an assumed selectiveness on the part of the bug by its opportunity for attack on either sex, which must be practically equal, or by a supposititious immunity of the male sex to pellagra infection.

Roaches, though common throughout the region, are negligible in connection with our subject unless the disease is found to be transmissible by means of contaminated foodstuffs. Should this be the case their rôle must still be subordinate to that of the housefly.

The family Tabanidae, which includes the well-known horse flies, should be mentioned because of their blood-sucking habits and the fact that at certain times and in some localities they attack man with a degree of frequency and persistence. These attacks are, however, desultory and have no part in the essential economy of the flies. In Spartanburg County, flies of this group were found to be far from common, and this fact together with their irregular attack on man, and an entire lack of association with him, serves to exclude them conclusively from consideration.

When attention was given to the fleas of the region, a somewhat unexpected condition was found to exist. Superficially considered, these insects might be thought to present possibilities in connection with pellagra transmission, and great care was taken to collect all possible information regarding them as well as material for study. So

uniform were negative statements as to attacks by them that we were forced to believe that as pests of human beings in the locality, fleas play but a small part. This is the less remarkable in view of the fact that we were unable to collect the human flea, *Pulex irritans*, at any time. The cat and dog fleas as well as a few chicken fleas, *Echinophaga gallinacea*, were collected from various hosts and a number of rats, captured in the town of Spartanburg, were infested by the European rat flea, *Ceratophyllus fasciatus*, and a considerable number of the Indian rat flea, *Xenopsylla cheopis*. A few specimens of *Ctenopsylla musculi* were also obtained.

The sharply defined host habits of most fleas render the species which are characteristic parasites of cats, dogs, rats, etc., rarely troublesome to man under normal American conditions. When conditions are favorable for the inordinate propagation of the cat or dog fleas or an epizootic decimates the host species, as in plague, this may occur, but under ordinary circumstances these fleas will remain on human beings a comparatively short time and transference from man to man probably occurs but seldom. Transmission of human disease by the same channel would similarly be unlikely to occur. In addition, the sex incidence of pellagra cannot be satisfactorily explained by the incrimination of these insects.

The only mosquitoes of the region studied which justify consideration in connection with possible pellagra transmission are the two house species, *Aedes calopus* and *Culex quinquefasciatus* (fatigans). The latter species is nocturnal in habit, and its incrimination is incompatible with the sex incidence of pellagra.

While the yellow-fever mosquito is emphatically a day mosquito, its occurrence in Spartanburg County is by no means constant or regular. It should be noted that in spite of its presence in the town of Spartanburg, the species was not taken in the country districts or at points remote from railroad communication with its more southern and regular habitat. In the summer of 1912 no individuals of this species were observed, while in 1913, from June 1, the *Stegomyia* was a common and troublesome pest in Spartanburg. At the time of its appearance in 1913, the seasonal epidemic of pellagra was well advanced and the disease was showing great activity. On no accepted theory as to its period of latency, whether of short or long duration, can this species be incriminated in view of these phenomena. In spite of its day-biting habits, therefore, *Aedes calopus* must remain excluded as a causative agent.

Our studies in 1912 convinced us that there was little evidence to support the incrimination of any species of *Simulium* in South Carolina in the transmission of pellagra. Reviewing the group as a whole, we

find that its species are essentially "wild" and lack those habits of intimate association with man which would be expected in the vector of such a disease as pellagra. Although these flies are excessively abundant in some parts of their range and are moderately so in Spartanburg County, man is merely an incidental host, and no disposition whatever to seek him out or to invade his domicile seems to be manifested. Critically considered, it is nearer the fact that usually man is attacked only when he invades their habitat.

As our knowledge of pellagra accumulates, it is more and more evident that its origin is in some way closely associated with the domicile. The possibility that an insect whose association with man and his immediate environment is, at the best, casual and desultory, can be active in the causation of the disease becomes increasingly remote.

Our knowledge of the biting habits of *Simulium* is not complete, but it is evident, as regards American species at least, that these are sometimes not constant for the same species in different localities. Certain species will bite man freely when opportunity offers, while others have never been known to attack him. To assume that the proximity of a *Simulium*-breeding stream necessarily implies that persons in its vicinity must be attacked and bitten is highly fallacious. In Spartanburg County attacks by *Simulium* seem to be confined to the immediate vicinity of the breeding-places. Our records and observations, exceedingly few in number, refer almost exclusively to such locations. Statements regarding such attacks, secured with much care and discrimination from a large number of persons, including many pellagrins, indicate conclusively that these insects are seldom a pest of man in this county. A certain number of the persons questioned were familiar with the gnats in other localities, but the majority were seemingly ignorant of the existence of such flies with biting habits. This is especially striking, in view of the fact that the average distance of streams from the homes of the pellagra cases studied was about 200 yards, many being at a distance of less than 100 yards, and that 78 per cent. of these streams were found to be infested by larval *Simulium*. Such ignorance in a large number of persons cannot be overlooked and indicates strongly that our belief in the negligible character of local attacks by *Simulium* is well founded.

In localities infested by "sand-flies," mosquitoes, etc., these pests are always well known and the ignorance described above is very significant.

Such positive reports as we received nearly always referred to bites received in the open, along streams, etc., and observations made of their attack were of those on field laborers in similar situations. Males engaged in agricultural pursuits are almost exempt from pellagra

in Spartanburg County. During the season of 1913, in some two or three instances, observations were made of the biting of *Simulium* and some additional and entirely credible reports were received. These observations and reports were under conditions identical with those referred to in the reports of 1912 and confirm the conclusions based on the observations of that year. I would repeat with emphasis that it is inconceivable that a fly of the appearance and habits of the prevalent species of *Simulium* could be present in such a region, especially about the haunts of man and attack him with sufficient frequency and regularity to satisfactorily account for so active and prevalent a disease as pellagra without being a well-known and recognized pest.

In connection with the conditions in the Piedmont region of South Carolina, it may be well to cite the results of a study of those in the arid region of western Texas.

In May, 1913, in company with Capt. J. F. Siler of the Thompson-McFadden Pellagra Commission, I visited the region of which Midland in Midland County is the center. This region is very dry and totally devoid of running water for a long distance in every direction. The only natural source of water-supply, a few water holes and ponds, were visited and found to be of such a nature that the survival of *Simulium*, far less its propagation in them, is absolutely impossible. The nearest stream affording possibilities as a source of *Simulium* is 60 miles away, while the average distance of such possibility is not less than 100 miles.

Artificial sources of water-supply were also investigated carefully and were found to offer no opportunity for the breeding of *Simulium*.

At Midland the histories of five cases of pellagra were obtained, which gave clear evidence that this place or its immediate vicinity was the point of origin. Persons of long residence in the country were questioned as to the occurrence of such flies as *Simulium* and returned negative answers. These included a retired cattle owner, who is a man of education and a keen observer, an expert veterinarian stationed in the country who has the cattle of the country under constant observation, and a practical cattle man, manager of a ranch and of wide experience. The latter had had experience with "buffalo gnats" in other localities (in the East) and is well acquainted with them. His close personal supervision of the cattle under his charge, makes it practically certain that he would have discovered these gnats had they been present in the country.

At the time the study was made, *Simulium* was breeding and active in the adult state in the vicinity of Dallas, Texas, in the eastern part of the state. We have here a region in which cases of pellagra have originated, yet in which *Simulium* does not and cannot breed. Dr.



Sambon has suggested that in the absence of *Simulium* certain midges of the family Chironomidae may assume the function of transmitting pellagra.

In the course of our field work, especial attention was paid to small flies of all kinds, and although the conditions were favorable for the discovery of any blood-sucking *Chironomidae* or other midges, none were collected during the two seasons spent in the field from early spring until late fall.

The reports opposed to the frequent attacks of *Simulium* may be taken as applying also to the present group. At the risk of repetition, I would note that in the coast region of South Carolina, "sand-flies" are abundant, and are only too well known to the inhabitants.

In the course of the work of 1912, we became convinced that *Stomoxys calcitrans*, the stable-fly, which had been regarded by us merely with suspicion, was an insect which merited the closest study in connection with pellagra transmission. It is practically cosmopolitan in distribution and is found at considerable altitudes and in high latitudes. It is an abundant species almost everywhere throughout its range, and in many places is a very serious pest of domestic animals. Under favorable conditions, there are sometimes outbreaks of this fly which cause the death of many animals and untold worry and suffering to all live stock within its influence. Primarily and by preference, it preys on the larger domestic animals and breeds in their excreta. Nevertheless, it attacks man frequently and with persistence, although with some irregularity, depending to a certain extent on the presence or absence of the animals on which it usually feeds and seemingly also on weather conditions. Its association with domestic animals brings it also into somewhat close association with man, and it readily takes up a more or less prolonged residence in and about human habitations. Ample corroboration of these statements occurs in the literature of the species.

The longevity of *Stomoxys calcitrans* in nature is not known with accuracy, but experimentally the fly has been kept in confinement and fed artificially for a period of eighty-nine days. This record was obtained by W. V. King in the course of pellagra transmission experiments in 1913. The average life of the flies used in the work was much less than this, and it is highly probable that the natural life is also much shorter than three months. This species frequently attacks several hosts during the taking of a single meal, and the habit is of importance in connection with disease transmission, especially when mechanical transference of an organism is possible. The habit seems to be less a matter of choice than because of the frequency with which it is dislodged by the animal attacked. When this occurs before the

appetite is satisfied, another spot on the same animal or another is selected and a fresh bite inflicted. Experimentally fed flies usually, when undisturbed, remain attached until the completion of the meal, unless the part selected is unproductive, when the proboscis may be withdrawn and another chosen. There is great variation in the time required for complete engorgement, depending apparently on the blood-supply of the skin at that point. On man, if applied to the lower extremities, a full meal may be taken in three or four minutes, while not infrequently the fly remains as much as fifteen minutes before voluntarily withdrawing the proboscis.

In passing, it may be noted that *Stomoxys* is purely predatory in its feeding habits; it is not attracted to such substances as the nasal secretions of animals nor to carrion or offensive substances other than the excreta of the larger herbivorous animals. It is reported to breed in the feces of hogs, but in my own experience I have not observed this. I have not seen them apparently attracted to hoggpens nor attacking hogs, though they doubtless do so on occasion. This species is distributed throughout the state of South Carolina, and in Spartanburg County it is very abundant. In all the cities and towns of the state it is present in large numbers and in the rural districts its abundance is even greater. Mill villages in or about which cattle are invariably kept are infested by large numbers of these flies and the usually unscreened houses are quite regularly entered by them. That the inhabitants are frequently bitten cannot be doubted as the overwhelming numbers of reports indicate. Some individuals can almost always be found in or about all houses in the mill villages of the region, and a favorite resting place is about the porches on which much time is spent by the inhabitants.

Many reports were received of attacks of *Stomoxys* on persons engaged in milking cows, and this duty falls largely on the female members of the household. A milk cow is kept by about one family in three, and the milking is done at the home of the owner to which the animal is brought, or in some mill villages, the cattle are excluded and milked and cared for in the common pasture.

When it is recalled that a high percentage of cases of pellagra occur among those who spend a large proportion of their time in or about the home, the habit of *Stomoxys* in frequenting not only the interiors of dwellings, but those parts of their exteriors which are occupied by the inhabitants is important.

Statements regarding the biting of man by *Stomoxys* were so universal in our territory and were so amply confirmed by our own experience and observations, it must be admitted that the habit is frequently practiced. It should be emphasized that man is not attacked

with the frequency and persistence displayed by such insects as the house mosquitoes, and it is not impossible that the distributional picture of pellagra may be largely accounted for by this fact. Given even a moderate degree of infectiousness and such a transmitter as the common species of *Culex*, the spread of the disease could hardly fail to be much greater and more rapid than it is known to be.

In addition to the reports received as to the biting habits of this fly, our own observations and the published statements regarding it, it seemed desirable to obtain, if possible, definite proof of the frequency with which human beings are attacked. By examination of the stomach contents of a large number of flies and determination of the species to which the host belonged it was hoped that some conclusion could be reached. The method adopted was the application of the precipitin reaction to the blood ingested by flies captured in localities where there would be reasonable opportunity for the selection by them of human hosts. More than 600 dissections were made, but the results in only about 200 of these are at present available. Of these, 109 were taken under circumstances which implied a fair chance for the fly to have recently attacked man, that is, in or about occupied dwellings, stores, etc. Six of these, or  $5\frac{1}{2}$  per cent., gave a positive human reaction.

In collecting the material no effort was made to select weather especially favorable for attack on man by the flies, and the days when captures were made it covered quite a wide range of meteorologic conditions.

The number of human reactions obtained may seem small, but that at one period of a few minutes at each spot, such a number of flies were found, weather disregarded, to have recently fed on human beings, appears to indicate a rather free exercise of the habit. Were it possible to capture and test all flies within the bounds of a mill village for a twenty-four-hour period, and should this ratio hold, the result from our point of view would be startling. As a matter of fact, the percentage would almost certainly fall below that mentioned and yet would, with equal certainty, represent a large number of bites with their attendant possibilities of disease transmission.

A point of interest in this series is that two of the six flies had fed on man only, three had fed also on cattle, while one gave a reaction with the sera of ox, horse and man. The blood of bovines only was determined in the stomachs of 80 per cent. of this series; of equines only, in but 6.4 per cent., while bovine and equine reaction was obtained in 7 per cent. The latter results are strongly corroborative of the observations on interrupted feeding by this species.

It has been suggested that the bite of *Stomoxys* is so painful to human beings that but a small percentage of the flies attempting to draw blood from them could succeed in doing so, and that almost all

would be driven off without attaining their object. Even if this were true it has been shown that trypanosomiasis may be communicated by the mere picking of the skin by an infected *Glossina*, even when the fly is immediately removed and no blood drawn. It may be assumed that if the parasite of pellagra is a protozoon and *Stomoxys* its carrier, that the same means may be effective.

It cannot be doubted that, almost invariably, the stable fly is driven from its human host when the pain of the bite becomes noticeable. To ascertain whether blood, even in small quantity might not have, by that time, been drawn, a series of tests was made in which clean-bred *Stomoxys* were allowed to bite selected parts of the bodies of several individuals. Such parts of the extremities as are frequently exposed were chosen and single flies confined in flat-bottomed shell-vials 25 by 100 mm., the end covered with gauze were applied to the bare skin, or in some instances the fly was allowed to bite through the stocking which covered the part. No hesitation was usually shown by the fly in proceeding to secure its meal, whether the skin was covered or bare. When the tube had been applied the subject was instructed to report the instant the first indication of pain was felt, the tube being then immediately removed and the fly dissected. Thirty-three flies were thus used, the forearm, lower leg and ankle being selected for the infliction of bites. In eleven instances, or 33 per cent. of the tests, before pain was felt, an amount of blood was drawn which ranged from one-third to a full engorgement, and in five, or almost half, a full meal was taken. In three cases on two subjects no sensation was felt at any time and the fly completed its feed and withdrew the proboscis without the knowledge of the host that a bite had been inflicted.

In many cases, the insertion of the proboscis and the early part of the process of drawing blood causes no sensation. At times when the fly is partially engorged the proboscis is thrust deeper or its position slightly changed when a more or less severe prick is felt. Were the bite incurred under natural conditions the victim would naturally assume that this was the moment of attack. The wary fly, in spite of partial engorgement, is usually able to withdraw the proboscis and avoid a hasty, ill-directed slap.

In the painless bites it is evident that anesthetic areas of the skin were selected, and analysis of my notes shows that one was inflicted one inch in front of prominence at lower end of tibia, one on external aspect of lower leg near median line and 6 inches above the ankle, the third at about the same point but 2 inches higher. This was on a different subject from the preceding.

Two other full engorgements, in which some pain was felt, were drawn from 1 inch posterior to the prominence at lower end of tibia, the other from 1 inch in front of the prominence. No painless bites

were inflicted on the forearms, but five blood-meals, ranging from one-third to two-thirds the full feed, were drawn from approximately the same areas as those already indicated.

It is of common experience that the stocking-clad ankle and parts of the leg adjacent thereto are favorite points of attack by *Stomoxys calcitrans*, even in the case of persons who are habitually shod. As far as these experiments go, they indicate that, from such parts of the body, an amount of blood may be drawn which by all analogy should be amply sufficient to cause the infection of the fly with any parasite present in it and capable of causing such infection.

House-flies are everywhere excessively abundant in Spartanburg County, houses are generally unscreened and if pellagra should prove to be communicable through contamination of food, utensils, etc., this ubiquitous pest will probably be found to play an important part in the spread of the disease.

Blow-flies are prevalent, and though far less numerous than house-flies, are, from their predilection for human excreta as a breeding-place, likely to be individually very active in such dissemination.

The facts which have come to light regarding sewage disposal by means of efficient water carriage and its seeming effect on the occurrence of pellagra, gives additional interest to the consideration of *Musca domestica* in this connection. Our present knowledge does not, however, justify a discussion of these facts or an attempt to determine whether the presence of sewers and the failure of pellagra to become active are merely coincidental and have no direct correlation; whether their effect is indirect or whether the presence of this system is a prime factor in the control of the disease. If the latter is the case, the incrimination of the house-fly seems certain.

#### CONCLUSION

Our studies have led us to believe that ticks, bedbugs, mosquitoes, fleas, horseflies, and, in the absence of further and more incriminating evidence, the lice, may be dismissed from consideration as transmitters of pellagra; that there is not only insufficient evidence to incriminate flies of the genus *Simulium*, but much evidence directly opposed to such incrimination and that the biting stable-fly, *Stomoxys calcitrans*, shows in marked degree those characteristics of distribution, habit and association with man which would pre-eminently fit it to be the vector of pellagra if transmission of the disease by a blood-sucking insect is shown to be possible.

If pellagra is found to be an intestinal disease of bacterial origin, house-flies and others of similar habits will in all probability be found to be an active factor in its causation.